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Effect of different levels of probiotics on rumen environment and microbial condition in local goat kids

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The present study aims to find out the effects of adding different levels of probiotics and concentrate diet on the rumen environment, volatile fatty acid production and bacteria counts of local Iraqi goat kids. This study was conducted in the field of a goat breeder in the area of Karmashi Karma Bani Saeed district, Souk Al-Shuyoukh district, Thi-Qar Governorate, Iraq. This study was conducted for 105 days, including 15 days preliminary period on 18 male kids of local goats at the age of 3 months and with an average weight of 16.17 ± 1.10 kg. The kids were randomly distributed to six treatments with two levels of concentrated feed (40, 60%) and three (3) levels of probiotics (0, 2.5, 5) g/head/d. The probiotics included *Lactobacillus acidophilus* 10^8, *Bacillus subtilis* 10^9, *Bifidobacterium* 10^8, and *Saccharomyces cerevisiae* 10^9. The group of kids fed 2.5 gm probiotic/ head/ day and 40 or 60% concentrate feed were significantly superior to the kids that were fed only 40% concentrate diets without the addition (52.49, 53.61, 43.77 mmol/L), respectively. The % of acetic and butyric acids, were not affected by the addition of the probiotic at different levels of the concentrate diet. The group fed (60%) concentrate diet and the probiotic (2.5 or 5 g/ head/ day) had the highest percentages of propionic~~%~~ (22.67, 22.33%), respectively, compared to the lowest percentages 17.71%, recorded by the kids fed 40% concentrate diet without probiotics. The pH of the rumen fluid was not affected fed different percentages of concentrate supplemented with two levels of the probiotic 2.5 or 5 g/head/day. While the level of NH_3-N was affected by the concentrated diet, kids ate (60% concentrate) recorded significantly higher levels compared to that fed (40% concentrate). Addition of probiotics with different levels of concentrate diets produced highest total bacteria counts >10.50× 10^9 CFU/ml before feeding and >18.50 × 10^9 CFU/ml at the third hour after feeding.
Keywords: Probiotics, conc feeding, vfas, goat kids. thi-qar governorate, Iraq.

INTRODUCTION

Goats are an important economic resource in small herd production systems, and feeding is the main component in goat husbandry, which has received special attention in improving the performance and reproduction of these animals. Many studies have been conducted to develop the benefit of fodder through the use of feed additives and prohibition of the use of antibiotics in some countries. The search for sources of alternative feed additives has begun in a large way, and probiotic is one of these alternatives (Seo *et al.*, 2010), which is known to be best used by animals.

Probiotics have been studied extensively in ruminants, including goats (Kumar and Jha, 2019). Studies have shown that probiotics improve rumen fermentation by increasing the production and absorption of volatile fatty acids (VFAs), reducing the production of ammonia, and improving the microbial balance in the rumen (Bach *et al*., 2019; Hosseini *et al.*, 2014). In addition, probiotics can help reduce the production of methane and other gases and improve feed efficiency. Probiotics also play a role in reducing the negative effects of dietary toxins and improving the health and performance of goats (Sun *et al*., 2021).

Herbivore and ruminal microorganisms have a symbiotic interaction in which the microbiota aids the animal in feed digestion and absorption to supply the host with energy and small molecule nutrients (Guo *et al.*, 2021). Rumen VFAs, which are generated by microbial fermentation of carbohydrates are absorbed and used by animals as a source of energy, have a major role in determining the pH of rumen fluid (Del Bianco *et al*., 2018; Franco *et al.*, 2020; Gleason *et al.*, 2022). In addition, the probiotic stimulates the growth of lactic acid-consuming protozoa (Al-Galibi, 2010; Hameed *et al.*, 2020), thus greatly increasing the efficiency of the rumen function.

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The present study aims to find out the effect of adding different levels of probiotics and concentrate diet on the rumen environment, volatile fatty acid production and bacteria counts of local Iraqi goat's kid.

MATERIALS AND METHODS

This study was conducted in the field of a goat breeder in the area of Karmachi, Karma Bani Saeed district, Souk Al-Shuyoukh District, Thi-Qar Governorate, for 105 days, including 15 days preliminary period. A total of 18 local male kids were offered in an individual feeding trial, with an average age of three (3) months and weight of 16.17 kg. Kids were divided into six nutritional groups (3 kids each), fed a concentrated diet consisting of (20% wheat flour, 25% wheat bran, 25% barley, 20% crushed corn, 7% soybean meal and 3% salts and vitamins) with rice hay treated with molasses and urea. The 1st diet group was control, 0 g probiotics/h/d in addition to (40% concentrate + 60% rice straw). The 2nd group was given 2.5 g probiotics/h/d in addition to (40% concentrate+ 60% rice straw). The 3rd group was given 5 g probiotics/h/d in addition to (40% concentrate+ 60% rice straw). The 4th group was given 0 g probiotics/h/d in addition to (60% concentrate+ 40% rice straw). The 5th group was given 2.5 g probiotics/h/d in addition to (60% concentrate+ 40% rice straw). The 6th group was given 5 g probiotics/h/d in addition to (60% concentrate + 40% rice straw). Please insert a Table 1 showing all diest and combination). The kids fed the concentrated diet as 3% of their body weight, chemical analysis of concentrate diets and rice straw is shown in Table 1. The kids were examined by the veterinarian and all veterinary procedures were taken throughout the study period, as the kids dosed against intestinal and hepatic worms using Al-Bendazole produced by the Italian company Doxal at a dose of 150 mg/kg live weight. The kids were also injected with Ivermctine (0.21 cm3 / 10 kg live weight) produced by the English company Norbrook, subcutaneously.

Table 1 showing different ration combinations and probiotics (treatments) added/offered.

The rumen fluid was withdrawn and the pH was measured using a digital pH meter 9900. The rumen fluid was filtered by a four folds cheese making cloth. After which the rumen fluid was withdrawn to measure the volatile fatty acids with the GC mas device. As for the bacterial count, 1 ml of rumen fluid was taken and 9 ml was added to it. Peptone water was added to make several dilutions, then it is cultivated on suitable media for each type of bacteria studied.

The statistical program SPSS (2012) was used to analyze the data using a single factor randomized design of six treatments and test for significant differences among means using the Revised Least Significant Differences (RLSD).

Table 1. Chemical analysis of experimental diets

Nutrients	Concentrate %	Rice straw %
Dry matter	92.00	88.50
Crude protein	15.89	4.90
Ether Extract	3.25	1.92
Crude Fiber	6.99	29.23
NFE	70.81	40.95
Ash	3.06	11.50
NDF	21.76	73.60
ADF	6.15	64.90
ADL	3.56	25.70
Hemicellulose	15.61	8.70
Cellulose	2.59	39.20

RESULTS AND DISCUSSION

***Volatile fatty acids*: ** The amount of total volatile fatty acids was significantly ($P<0.05$) affected by adding different levels of probiotic (2.5 or 5) gm probiotic/head/day (Table 2). As the group of kids that were added to their diets 2.5 g probiotic/h/d and (40 or 60%) concentrated feed recorded a significant superiority ($P<0.05$) over the group of kids that were fed only (40%) concentrated diets without addition of probiotics (52.49, 53.61, 43.77 mmol/L, respectively). While the addition of 5 gm probiotic/h/d to diets containing (40 or 60%) concentrated diet, and the group that consumed (60%) concentrated diet only recorded (47.12, 51.17, 49.58) mmol/ liter for the three treatments, respectively. Which reflects that the addition of the probiotic to the diets that contain 40 or 60% concentrate produce the same level of volatile fatty acids, and therefore it is possible to reduce the percentage of concentrated diets in feeding the kids, which makes the ration economical.

These results are consistent with what was obtained by Cai *et al.* (2021) when feeding the Chinese goats with probiotics at different levels, and they found that the levels close to (2.5%) produced the highest levels of total volatile fatty acids in the rumen (55.01 mmol/ L).

The percentage of fatty acid, acetic acid and butyric acid, was not affected by the addition of the probiotic at different levels or by the difference in the percentage of the concentrated diet. While propionic acid% was affected significantly ($P<0.05$) by adding the probiotic and the percentage of concentrated diet, as the group that was fed (60%) concentrated diet supplemented with probiotic (2.5 or 5 g/head/day) had the highest percentages of this acid (22.67, 22.33%, respectively), compared to the lowest percentages shown by the group of kids that was fed 40% concentrated diet without the probiotics (17.71%). The reason may be due to the increase in the proportion of the concentrated diet that contains dissolved and available carbohydrates with a high percentage of glucose, which is the raw material for the production of propionic acid. In spite of the decrease in the percentage of the concentrated diet to (40%), the addition of the probiotics modified the

Table 2. Total volatile fatty acid (VFAs, mmol/L) and percentages (%) of acetic, propionic and butyric acids of different treatments

Treatments	Total fatty acid	Acetic acid % (A)	Propionic acid% (P)	Butyric acid %	A/P ratio
T1	43.77±4.62b	69.64±5.12a	17.71±2.18b	17.00±1.58a	3.59±0.09a
T2	52.49±4.90a	64.35±5.14a	18.12±2.17ab	16.53±1.64a	3.55±0.10a
T3	47.12±4.95ab	66.98±5.17a	18.00±2.25ab	15.00±1.42a	3.72±0.13a
T4	49.58±4.10ab	60.55±5.13a	20.11±2.23ab	17.34±1.55a	3.01±0.08b
T5	53.61±4.12a	62.13±5.18a	22.67±2.28a	15.20±1.40	2.74±0.06c
T6	51.17±4.87ab	62.00±5.11a	22.33±2.20a	15.47±1.46a	2.78±0.06c

*T1 = 40% concentrate + 0 probiotics, T2 = 40% concentrate + 2.5 probiotics, T3 = 40% concentrate + 5probiotics, T4 = 60% concentrate + 0 probiotics, T5 = 60% concentrate + 2.5 probiotics, T6 = 60% concentrate + 5 probiotics * Means in a same column with different letter differ significantly at 0.05 level.

proportion of propionic acid and made it not significantly differ from the groups of kids fed (60%) of the concentrated diet.

Cai *et al.* (2021) found an increase in propionic acid concentration with an increase in the level of probiotic addition. Although the effect of the probiotic on the volatile fatty acids produced in the rumen is still not entirely clear, some researchers have found that feeding the probiotic to small ruminants causes an increase in the production of volatile fatty acids (Sadiek and Boehm, 2001; Abd El-Ghani, 2004). The increase in the concentration of volatile fatty acids in the rumen may be due to the decrease in methane production and the lack of energy loss that would be spent on the production of fatty acids (Williams and Newbold, 1990). However, some studies found a significant decrease ($P < 0.05$) in the production of volatile fatty acids in the rumen in growing lambs or mature goats fed diets to which the probiotic was added (Kowalik *et al.*, 2011), while the third group of researchers did not find a significant effect for probiotics in the production of volatile fatty acids (Soren *et al.*, 2013).

The ratio of acetic acid to propionic acid (A/P) was affected by increasing the concentrated diet ratio from 40 to 60% and adding the probiotic (2.5, 5g /h / d). The group fed (60%) recorded concentrate plus (2.5 or 5 gm probiotics) revealed a significant decrease ($P<0.05$) this percentage (2.74, 2.78, respectively) in comparison with rest of the groups. While the kids that were fed 40% concentrate diet only or the addition of the probiotic with its different concentration recorded that there was no significant difference (3.55-3.72%). This result confirms that the diets contain a percentage of roughage fodder (60% rice straw) produce high levels of acetic acid compared to feeds that contain less roughage (40% rice straw).

***pH and NH3-N*:** The pH of the rumen fluid was not affected by the different percentages of concentrated diet supplemented with two levels of probiotic 2.5 or 5 gm/h/d (Table 3). The pH value reflects the conditions of fermentation in the rumen, which is greatly affected by the type of diet and the percentage of concentrated diet. It is known that the addition of the probiotic, especially yeast, with high pH levels, therefore, the synergistic action between the probiotic and the proportion of concentrated diet led to maintaining the natural pH level suitable for the growth of organisms (5.8–6.2).

The level of NH_3-N was affected by the percentage of the concentrated diet, and the groups of kids that ate (60%) of the concentrated diet recorded significantly higher levels ($P<0.05$) compared to the groups that were fed (40%) of the concentrated diet, despite the addition of the probiotic at different levels, but it did not affect significantly within the same feed group. Increasing the percentage of concentrated diets in the feed provides high concentrations of protein decomposition products in the rumen, especially ammonia nitrogen, which is absorbed through the rumen wall and transmitted by the blood to the liver, which converts it into urea. It returns again to the rumen in the form of urea.

It was found that probiotics are effective in improving the anaerobic environment in the rumen, stabilizing the pH of the rumen, and providing nutrients needed by ruminants (Chiquette *et al.*, 2012). It may be considered an antioxidant with immunity improvement (Wang *et al.*, 2018). No change occurred in the pH in the current study as a result of adding the probiotic, while it changed significantly when adding *C. butyricum* (Cai *et al.*, 2021). The reason is due to the type of probiotic bacteria in raising or lowering the pH (Qadis *et al.*, 2004), and the action of the bacteria may be in preventing the decrease in pH in the rumen by decreasing the production of lactic acid and increasing its consumption by the microorganisms in the rumen (Chiquette *et al.*, 2012).

As for ammonia, the results of the current study agree with the results of the study (Cai *et al.*, 2021). The concentration of ammonia nitrogen in the rumen is affected by the addition of the probiotic, Fadel Elseed and Abusamra (2007) and Oeztuerk and Sagmanligil, (2009) found a significant increase ($P<0.05$) in the concentration of ammonia nitrogen as a result of the addition of yeast, and the increase in the concentration of ammonia nitrogen may be due to the improvement of the activity of microorganisms in the rumen, which analyze protein into carbon and ammonia (Kiran and Kumar, 2013).

Table 3. pH and NH_3-N concentration in the rumen of kids fed 40 or 60% concentrate supplemented with 2.5 or 5 gm/h/d

Treatments	pH	Ammonia (mg/100ml)
T1	6.12±0.55a	16.70±0.35b
T2	6.54±0.53a	17.16±0.34b
T3	6.61±0.63a	17.22±0.34b
T4	5.98±0.54a	18.11±0.35a
T5	5.43±0.58a	18.57±0.37a
T6	5.67±0.56a	18.76±0.36a

*T1 = 40% concentrate + 0 probiotics, T2 = 40% concentrate + 2.5 probiotics, T3 = 40% concentrate + 5 probiotics, T4 = 60% concentrate + 0 probiotics, T5 = 60% concentrate + 2.5 probiotics, T6 = 60% concentrate + 5 probiotics. *Means in a same column with different letter differ significantly at 0.05 level.

***The rumen bacteria count*:** The total bacteria, lactic acid bacteria and cellulolytic bacteria counts were affected significantly ($P<0.05$) by adding the probiotic (2.5 or 5) g/h/d to diets containing (40 or 60%) of the concentrated diet at the time before feeding or after three hours of feeding (Table 4). There was a significant increase ($P < 0.05$) in the number of total bacteria at zero or three hours when adding the probiotic at the level of 5 gm/h/d and a diet containing 40% concentrate. The group of kids fed 40% concentrated ration with 5 gm/h/d probiotic, 60% concentrated feed with 2.5 gm/h/d, and 60% concentrated ration with 5 gm/h/d found the highest total number of bacteria exceeded (10.50×10^9 CFU/ml) at the time 0 hours and more than (18.50×10^9CFU/ml) at the 3rd hour after feeding. It appears from the numbers of total bacteria and lactic acid bacteria after three hours of feeding were higher for all diets compared to their numbers before feeding. While the cellulolytic bacteria were affected significantly ($P<0.05$) by the difference in the percentage of concentrated diet, especially after three hours of feeding, it increased with the decrease in the percentage of concentrated diet.

The reason behind the increase in total, cellulolytic bacteria and lactic acid bacteria as a result of the addition of the probiotic may be due to the availability of a suitable environment for the growth of microorganisms and the best possible fermentation. The probiotics stabilize the pH values in the rumen with an increase in the numbers of total and cellulolytic bacteria (Al-Galibi., 2010). It also works to increase the decomposition of fibers and organic matter in the diet, which leads to an increase in the energy produced necessary for the growth of microorganisms, and then an increase in the amount of microbial protein produced (Tripathi *et al*., 2008; Al-Galibi. , 2010).

***Conclusion*:** The findings showed that using probiotics (either 2.5 or 5%) along with 40% or 60% roughage could increase the concentration of volatile fatty acids and the number of bacteria in the rumen. Probiotics should be used when feeding local goats up to 60% of poor roughages.

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***Consent to participate*:** All agree to participate.

***Consent for publication*:** All agree for publication.

Table 4. Total bacteria, lactic acid bacteria and cellulolytic bacteria count (CFU/ml) of rumen fluid of kids fed 40 or 60% concentrate supplemented with either 2.5 or 5 gm/h/d.

Treatments	Total Bacteria x10^9		Lactic acid Bacteria x10^7		Cellulolytic Bacteria x10^7	
	0 hour	3 hours	0 hour	3 hours	0 hour	3 hours
T1	6.52±0.66C	14.66±1.46b	5.62±0.43b	7.45±0.70c	3.72±0.29b	5.20±0.32a
T2	10.00±1.00Ab	16.87±1.38ab	5.99±0.41b	8.49±0.72ab	4.99±0.30a	4.78±0.33a
T3	10.91±1.10A	19.21±1.59a	8.00±0.42a	8.66±0.71a	5.17±0.38a	4.55±0.34ab
T4	8.68±0.86B	16.32±1.47ab	5.73±0.44b	7.86±0.69b	3.16±0.28b	4.00±0.33b
T5	10.87±1.10A	18.53±1.50a	6.00±0.45b	8.98±0.73a	3.28±0.25b	4.22±0.20b
T6	10.77±1.10A	19.00±1.52a	5.91±0.44b	8.77±0.70a	3.46±0.26b	4.31±0.20b

*T1 = 40% concentrate + 0 probiotics, T2 = 40% concentrate + 2.5 probiotics, T3 = 40% concentrate + 5 probiotics, T4 = 60% concentrate + 0 probiotics, T5 = 60% concentrate + 2.5 probiotics, T6 = 60% concentrate + 5 probiotics. *Means in a same column with different letter differ significantly at 0.05 level.

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